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REDUCTION DIVISIONS IN THE POLLEN MOTHER CELLS OF ALLIUM TRICOCCUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 215

MILDRED NOTHNAGEL

(WITH PLATES XXVIII–XXX AND ONE FIGURE)

Introduction

In accordance with a suggestion of Dr. D. M. MOTTIER made while the author was attending Indiana University during the summer of 1914, material showing the reduction divisions of *Allium tricoccum* was examined with the idea of comparing it with the results of a previous investigation of *Allium cernuum* (**24**). In many stages the results were the same, but a further investigation of other phases of the spore mother cell development has forced the author to change some views previously held.

The nuclei are large and the haploid number of chromosomes is only 8; consequently, individual members or portions of the spirem can be traced through various stages with less difficulty than is usually encountered.

Literature shows a great diversity of opinion as to whether or not the chromosomes visibly retain their individuality throughout the resting period; also concerning the structure of the resting nucleus; the state of the chromatin as it enters the synaptic ball; the actual time and method of reduction; and the origin of the spindle fibers. These questions the author has attempted to answer.

Material and methods

The material was collected along the steep, damp, shady banks of Clear Creek, 6 miles south of Bloomington, Indiana. Collections were made 4 days and 5 days apart, from June 30 to July 27, and while some of the anthers at the earlier date failed to show differentiation of sporogenous tissue, many from the later collections were in the shedding conditions. Often all stages from resting to tetrad would be found in a single umbel.

Strong chromo-acetic acid was used for killing and fixing, and allowed to act 36 hours, the fluid being changed once or twice during that period, after which the material was washed 18–24 hours by means of repeated changes of water, slowly dehydrated, cleared in chloroform, and then imbedded in 52° paraffin.

Sections were cut 5–12 μ thick, varying with the stages sought. For the spindle and in some instances for the spirem, Flemming's modified triple stain was found to be preferable, although for all other phases, especially the early prophases, Haidenhain's iron-alum-haematoxylin gave the best results, lichtgrün in clove oil occaşionally being used for a counter-stain.

Description

PRESYNAPTIC AND SYNAPTIC STAGES

An attempt to determine the structure of the resting nucleus and the origin of the various stages that follow by first studying that resting condition would be difficult and indefinite. The investigation must begin with a stage concerning which there is little dispute, and from this may be traced the subsequent steps. Such a condition is to be found at late telophase of the last division in the sporogenous tissue, even though the chromosomes are more or less united by anastomoses, caused by considerable enlargement of the nucleus which followed the close association at early telophase (fig. 1). While still retaining this distinct individuality, although united with one another by anastomoses and at the same time slowly approximating end to end, a series of vacuoles appear along the median longitudinal portion of each chromosome (figs. 1–4), this being first made apparent by chromatin staining fainter

along that portion (figs. 2, 3). In the beginning the vacuoles are short, narrow slits (figs. 2, 3), but later, owing to their enlargement,

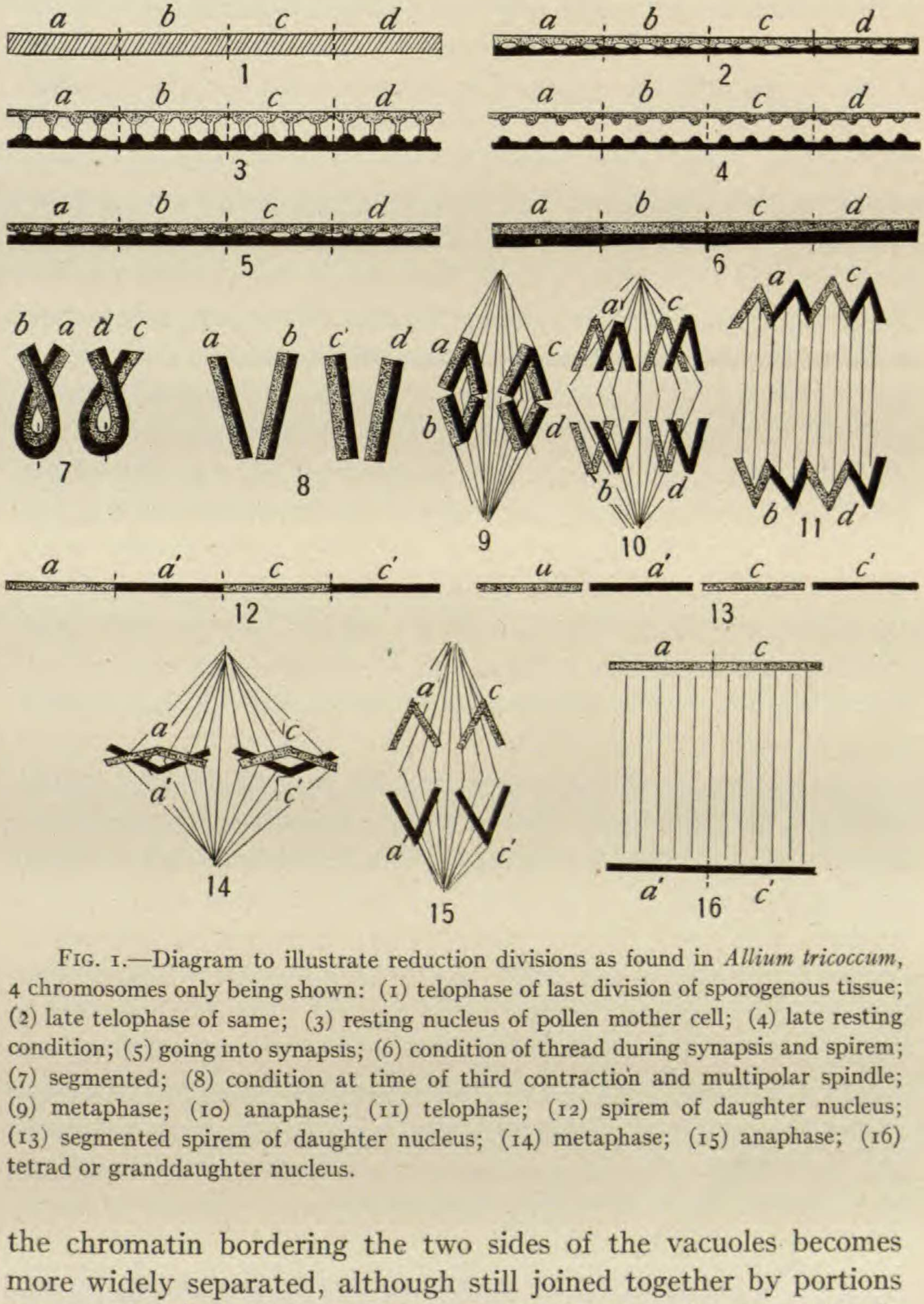

FIG. 1.—Diagram to illustrate reduction divisions as found in *Allium tricoccum*, 4 chromosomes only being shown: (1) telophase of last division of sporogenous tissue; (2) late telophase of same; (3) resting nucleus of pollen mother cell; (4) late resting condition; (5) going into synapsis; (6) condition of thread during synapsis and spirem; (7) segmented; (8) condition at time of third contraction and multipolar spindle; (9) metaphase; (10) anaphase; (11) telophase; (12) spirem of daughter nucleus; (13) segmented spirem of daughter nucleus; (14) metaphase; (15) anaphase; (16) tetrad or granddaughter nucleus.

the chromatin bordering the two sides of the vacuoles becomes more widely separated, although still joined together by portions or strands of chromatin that separate the vacuoles, resulting in

each chromosome having a ladder-like appearance (figs. 2–8). The rate of vacuolization is by no means uniform, as will be seen from an examination of figs. 2–5, and the distribution of chromatin over the ladder thread is very uneven, being heavier at the junction of the sides and the connecting strands. During this process several nucleoli appear in the comparatively large open spaces of the nucleus, each large nucleolus containing a relatively large oblong vacuole (figs. 5, 8).

It must be kept in mind that the ladder structures with their two sides and connecting strands have arisen from single somatic chromosomes, and are the result of a series of vacuoles along their median longitudinal axis. The so-called typical resting stage consists entirely of these structures, therefore, still joined more or less with one another by the anastomoses that arose at the last telophase (figs. 5–8). In the very early prophase the sides of the ladder are very fine (figs. 3, 4), but as the chromatin material increases, the threads become heavier and more uniform (figs. 5–8), while at the same time the anastomoses break down, and the connecting strands of chromatin between the two parallel sides grow finer and finer (figs. 7, 8) until they entirely disappear, leaving as a result two parallel threads (fig. 8), which are daughter halves of single somatic chromosomes.

As the parallel spirems, now entering synapsis, contract and condense, they gradually approach each other and approximate, at times twisting about one another (fig. 9) and resulting in a single thick spirem (figs. 10–13) which is the condition of complete synapsis, the heavy thread appearing as a homogeneous structure twisted and coiled about itself (figs. 11, 12), causing the mass to have a lumpy, granular appearance (fig. 12). At no time during this period, when sectioning and staining have been the best, is the thread lost to view (figs. 9–14); for even at the time of the greatest contraction, portions of the thread may be traced across the mass (fig. 11). This step in development is not necessarily uniform, for portions of the spirem in synapsis have been observed showing the double nature distinctly (fig. 10).

While these activities are going on, the nuclear cavity as well as the entire cell enlarges; although most of the growth is after the

chromatin material has contracted considerably. As the result of measuring many nuclei, only those being measured in which the entire nucleus was contained in the one section, the following statement may be made safely. Since the diameter of the contracted mass is considerably less than that of the nucleus just previous to contraction, or of the resting nucleus (figs. 7–12), synapsis is a true contraction. The synaptic mass lies almost or entirely against one side of the nuclear cavity, with the nucleolus just outside or partially held by a few strands of the spirem.

FORMATION OF BIVALENTS

As the synaptic mass loosens up, a comparatively thick, smooth thread may be seen twisting and winding about; and gradually loops free themselves, extending for various distances into the cavity (figs. 13, 14). It was stated that "a comparatively thick, smooth thread" was freed from the mass; while in fact the thread is irregular in outline and stains irregularly, the darker places being those denser chromatin aggregations seen in the ladder-like structure of the resting nucleus (figs. 3–9).

The spirem, although double in nature, is composed of single somatic chromosomes placed end to end, the double nature having arisen by the vacuolization or splitting of single chromosomes during late telophase of the last division of the sporogenous tissue.

When the spirem is entirely disentangled, loops of varying lengths are at first irregularly distributed within the nucleus (fig. 15); later, when they are peripherally arranged, the loops are longer and may be traced even entirely around the cavity, forming what is known as the hollow spirem (fig. 16). The double nature is rarely discernible during this latter period, since the thread has condensed and grown until it appears as a homogeneous structure staining uniformly throughout. Occasionally the halves of the thread separate (fig. 16) and may be seen even to twist about each other, the origin being in early synapsis (fig. 9, left side).

At the close of the hollow spirem stage the thread thickens and becomes entangled in the center (fig. 17), continuing until the typical second contraction results; which phase consists of the well known radiating loops that usually extend to the periphery

(fig. 18). The loops represent somatic chromosomes end to end which separate at the outer bend; that is, at the curve away from the entangled mass (figs. 19, 20, 21) rather than at the lower bend. Each radiating loop, therefore, does not represent the bivalents as seen in figs. 21*x*, *y*, 22. Instead, one arm of a loop pairs with and twists about an arm of a neighboring loop, this being plainly evident in the material. Fig. 19 illustrates such a condition, for here *a* and *a'* were formerly a continuous loop, likewise *b* and *b'*; but, following separation, *a* and *b* twist about one another, then opposite ends in all probability form a loop, resulting in *a*+*b* forming a bivalent, as seen in figs. 21*x*, *y*, 22, 23.

The bivalents of the aggregation continue to crowd together until only paired free ends extending from a dense mass can be recognized (fig. 20). When these segments loosen up, the chromosomes are found to be growing shorter and thicker. In case a misunderstanding should arise concerning the transition from fig. 21 to fig. 22, separate chromosomes have been drawn (figs. 21*x*, 21*y*). After the bivalents become dissociated and well distributed, eight are readily counted, each one representing 2 somatic chromosomes that formerly were end to end, although now they are twisted about each other. At times the 2 members of the bivalent are still continuous, that is, forming a loop; while in other cases they are not, causing the bivalent to be open at both ends. In either case it is not due to the splitting or to a separation of the halves of the spirem thread, since that spirem thread is composed of 16 somatic chromosomes with an end to end arrangement.

FROM THIRD CONTRACTION TO DAUGHTER NUCLEUS

About the time the bivalents are more or less distributed within the nuclear cavity, the fibers, which stain blue with Flemming's triple stain, appear outside the nuclear membrane (figs. 22, 23), running parallel to it, the ends extending out into the cytoplasm into which they merge (figs. 23, 24). While these are increasing in number and the nuclear membrane is disappearing, a peculiar behavior of the chromosomes has been noticed which seems to have a definite relationship to phases in the development of the spindle. The nuclear membrane slowly and unevenly disappears (fig. 23),

this being accompanied by further growth of fibers. These fibers gradually appear within the nuclear space even before the membrane is entirely gone (fig. 24). When this activity first begins, the chromosomes move slowly toward the center, so that by the time of the stage shown in fig. 25, where the membrane has entirely disappeared, the bivalents are tightly massed in the center, forming a *third contraction*. Step by step the kinoplasmic fibers encroach upon the chromosomes (figs. 25, 26), until they come in contact with them (fig. 27), this being immediately followed by the loosening up of the aggregation (figs. 28, 29). By this time there is a strong multipolar complex, which is apparent as early as the disappearance of the membrane. The number of poles may be many and irregularly arranged (figs. 28–31), and as the fibers are rearranged into sharper points, the chromosomes become more and more dissociated (figs. 28–31) and scattered upon the fibers. Those bivalents which formerly consisted of the two end to end somatic chromosomes and formed a loop (fig. 22) have separated transversely, making 16 chromosomes or 8 pairs (figs. 29, 30, 30*x*). Fastened to each member of a pair, approximately at the middle, is a group of kinoplasmic fibers (fig. 30*x*) extending to one of the several poles. As the multipolar spindle changes to the bipolar shape, the chromosomes that formerly were scattered irregularly upon the fibers (figs. 29–32) gradually arrange themselves upon the spindle's equator (figs. 32–35). During this latter period the chromosomes shorten considerably, having reached their largest size during multipolar phase, as well as slowly untwisting.

The forms that the pairs may assume now are various; some remaining slightly twisted, others form a U, while still others may become linked with one another, forming an X, or the ends of the pair may remain in contact, forming a ring. No form was found to be conspicuously dominant (fig. 31). Polar views of the spindle first show the chromosomes not to be peripherally placed (fig. 32), although before metaphase is completed they take that position (fig. 33). At this time the chromosome count again can be taken without difficulty.

The individual fibers that are attached to each chromosome may become so closely associated at the point of union with them

that the collection at first sight appears as a homogeneous mass and a part of the chromosome (fig. 34). These wefts retain their individuality for their full length and many times terminate in a very blunt pole (fig. 34).

During metakinesis, that is, just at the time when the paired segments separate, the dissociation of the two approximated halves, which originated in the resting stage (figs. 1–11), is finally completed (fig. 35), showing that the splitting or vacuolization of the somatic chromosomes in early prophase was in preparation for the homotypic division. There are now 16 chromosomes, for each half becomes entirely separated from its partner and does not again approximate (figs. 36–39). As the 16 members approach the poles, they shorten considerably, and as they crowd together an end to end formation results, thus forming an irregular spirem 16 chromosomes in length (figs. 37, 38). The crowding together continues until the entire chromatin mass is so closely associated that it is very difficult to distinguish the individual members; although, after the new nuclear membrane is formed, the looping spirem is again easily recognizable (fig. 39).

HOMOTYPIC MITOSIS

After the new membrane has been formed and the chromatin mass loosened up (fig. 39), anastomoses between portions of the looping spirem are everywhere evident. A coarse reticulum is formed as a result of the continual growth of the nucleus and the chromatin (fig. 40); but a resting stage is never reached. The spirem, which consists of 16 segments arranged end to end, will come directly from this reticulum (figs. 41, 42). This continuous spirem is quite regular in outline at first (fig. 41), but as the time for segmentation and formation of the multipolar complex approaches, the thread is more irregular and the outlines of the segments evident (figs. 42, 43); although it is not until the bipolar spindle is visible that the spirem separates into the 16 chromosomes (fig. 44). Simultaneous with this separation there is a pairing; so that 8 pairs of chromosomes come to lie upon the spindle fibers (figs. 44, 45). The paired chromosomes do not take the characteristic forms of the heterotypic mitosis, for at this time, although

there is a pairing, there is no twisting about of the members; but, instead, the tendency is to assume more elongated forms, such as hooks, rods, or wide U's. One member of each pair passes to a pole, where again there is an end to end approximation, as in heterotypic mitosis, of the 8 chromosomes or the haploid number (fig. 49). The tetrad, or 4 granddaughter nuclei, each containing 8 chromosomes, may all lie in one plane, although at times they occupy two planes.

Discussion

PRESYNAPTIC AND SYNAPTIC STAGES

Since the investigation began with the examination of the late telophase of the last division of the sporogenous tissue while the individual chromosomes were still plainly evident, although joined together by anastomoses, it is clear to the author that there is not a pairing of somatic chromosomes in fig. 1, or that a previous approximation has taken place. If such were the case, 8 chromatin groups only would be visible. Even at this early phase the vacuoles are making their appearance along the median longitudinal line. The chromatin bordering these vacuoles could not be called linin, although the later enlargement of these forms the fine chromatin structure (figs. 2–8) that ALLEN (1), MOTTIER (**21, 22, 23**), STRASBURGER (**30, 31**), DIGBY (**7**), and others term linin. Furthermore, the heavier masses of chromatin granules are due to the nature of vacuole formation; for, as they enlarge, a greater amount of chromatin will be left at the angles between the vacuoles, thus giving rise to the so-called "chromomeres" strung along at irregular intervals on a linin thread.

BEER (**2**), MOTTIER (**22, 23**), and MOTTIER and NOTHNAGEL (**24**) find a single spirem formed from the network, in which condition it enters synapsis; while ALLEN (1), GRÉGOIRE (**16**), GRÉGOIRE and WYGAERT (**17**), BERGH (**3, 4**), YAMANOUCHI (**32, 33**), ROSENBERG (**27**), and OVERTON (**25, 26**) see a pairing of spirems either previous to or during synapsis, this act involving the pairing of somatic chromosomes, presumably maternal and paternal, after which they approximate and at metaphase of heterotypic mitosis separate. Nothing more than an inference or a suggestion has

been found in the literature showing that the double nature of the spirem of the heterotypic mitosis is due to the splitting or vacuolization of somatic chromosomes in the early prophase. In a paper on *Galtonia* by Miss DIGBY (7), the split for the division at metaphase of the somatic mitosis is shown to arise by the vacuolization in early prophase, thereby forming the split or the double thread. A similar condition is found in *Vicia Faba* by SHARP (28), and by FRASER and SNELL (12). As the result of the conditions found, Miss DIGBY says: "By taking a broad and comparative view of this heterotypic prophase in relation to the somatic prophase, one is forced to admit that the parallelism of the one is homologous with that of the other"; although in her conclusion she states that "the parallel portions in both represent longitudinal halves of somatic chromosomes, and are probably sister halves of the same chromosome, which are now severally coming together and condensing to form the somatic or univalent chromosome." The series of drawings are incomplete at this critical period, and any conclusion would have to be based upon her drawings 39*a* and *b*, and 40, none of which is later than the writer's figs. 3 and 5. Furthermore, steps illustrating the origin of these figures from figs. 30 and 31 have not been shown, and the gradual transition from the "beaded" resting nucleus (DIGBY 7, figs. 33, 36, 37) to the double condition of the spirem after synapsis appears to be more of a theory or an inference than a statement of observed facts.

In the early prophases of the heterotypic mitosis of *Vicia Faba*, Miss FRASER (11) finds conditions corresponding to those observed in *Allium tricoccum*. In this paper she describes diamond-shaped meshes that are due to the splitting of the somatic chromosomes in the early prophases; then later, that is, in synapsis, the cross-connections between the meshes breaking down, thus forming the spirem; and finally, in early anaphase, each chromosome splitting preparatory to homotypic mitosis, the origin of the split having been seen in the diamond meshes. The idea is very similar to that described by the author, but as in the paper by Miss DIGBY (7), Miss FRASER fails to have a series sufficiently close to demonstrate the origin of the split and the formation of the spirem from these

meshes. While the resting nucleus is said to consist of diamond-shaped meshes, it is in all probability the same as the ladder-like formation of *Allium tricoccum*.

As has been previously stated, the number of chromosomes in *Allium tricoccum* is small and the size large, so that the difficulty so often encountered in following the development is considerably lessened. Throughout these critical phases every precaution has been taken to prevent overlooking important stages. Since the pollen mother cells at the upper end of a loculus are a little earlier than those at the lower end in development, at least two consecutive stages could be found in a single section. In every case the later of the two stages observed has been found in the upper end of the loculus, where the third has been found and drawn, decreasing to a great extent the possibility of omitting critical stages or placing the wrong interpretation upon the origin of the double character found in the resting nucleus. Had figs. 5, 7, or 8 been the first nuclei observed after the telophase of the previous division, the conclusion could readily be drawn that the double thread arose by the pairing of somatic chromosomes; but, after seeing the beginning of vacuolization (figs. 1, 2) and following its development step by step (figs. 2–9), no other conclusion is possible than that the paired threads going into synapsis are the two halves of single somatic chromosomes, and not paired somatic chromosomes, as held by GRÉGOIRE (**16**) and YAMANOUCHI (**32, 33**).

Fig. 8 illustrates the breaking down of the connecting strands between the two sides of the ladder-like structures, and on the right side of this figure the act has been completed, leaving the halves completely separated except for the portion connecting them at the end. MOTTIER (**22**) states that "the delicate threads joining the chromatic masses may be found lying close to each other and parallel, but this does not signify that a double spirem is in process of formation"; although in his figures of this stage (MOTTIER **22**, figs. 1, 17, 34) he shows nuclei very similar to that in fig. 5 of *Allium tricoccum;* and by tracing this further it is very probable that the origin of the double nature of the hollow spirem, as described by MOTTIER (**21, 22, 23**), might be found.

No suggestion of the spiral arrangement with the strands radiating from a "Chromatinknoten" as described by BONNEVIE (5) has been observed.

That the chromosomes retain their individuality and do not break up into a network is claimed by GRÉGOIRE (15), YAMANOUCHI (32, 33), SHARP (28), OVERTON (25, 26), STOUT (29), BONNEVIE (5), and LAWSON (19); this is also evident in *Allium tricoccum*. Although the chromosomes become considerably vacuolate and thus cause the net appearance, the individual members never lose their entire individuality, as can be seen from the description and drawings, and later, owing to a larger amount of chromatin material and a more even distribution of it, the chromosomes form a more or less continuous thread, the spirem.

Little growth occurs during the later stages of the resting nucleus, but when the chromatin mass starts to contract, it increases slightly in size, but not to the extent claimed by LAWSON (19); that is, that the appearance of the contracted mass is due to the growing away of the nuclear membrane. Comparison of figs. 8, 9, 10, and 11[1] will make this clear, as fig. 11 is a drawing of an entire nucleus, the dimensions of the chromatin mass being considerably less than that of figs. 7 or 8. It is not until the mass has contracted extensively that the large increase in size of the nuclear cavity occurs (figs. 10, 11). Were it but an apparent contraction, as LAWSON (18) states, due to the inflow of karyolymph into the nuclear cavity, the osmotic pressure would be decreased, not increased, and the increase in size would be the result of the larger amount of fluid that it must hold.

As synapsis approaches, the two halves of the spirem gradually approximate (figs. 9, 10, 11), the final step usually being accomplished during synapsis (fig. 11); although even as late as the stage shown in fig. 10 the act might not have been completed along the entire length. This process corresponds to that described by GRÉGOIRE (14, 15), YAMANOUCHI (32, 33), OVERTON (25, 26), and ALLEN (1), although these investigators claim it

[1] Figs. 1–12 were made with a magnification of 3500, while fig. 12 has a magnification of 2650, so that comparison of the latter with the former is not to be made where questions of size are concerned.

to be the approximation of whole somatic chromosomes, or pseudo-reduction.

FORMATION OF BIVALENTS

With close observation during early spirem, the double nature is still discernible, although it is rare that the two halves separate as found in *Allium cernuum* (MOTTIER and NOTHNAGEL **24**), and as commonly found in *Lilium*. The lumpy condition of the thread at this time is due to the larger chromatin collections of the early prophase (figs. 5–9), this appearance being the basis of the statements of FARMER and MOORE (**9**), MOTTIER (**22**), and others who interpret such as dividing chromomeres, thereby initiating the longitudinal split. This dual nature has been seen in most cases, although it has been attributed to two sources. Those believing in pseudo-reduction in early prophase assert that it is the two spirems that have paired, while MOTTIER (**21, 22, 23**), STRASBURGER (**30, 31**), BEER (**2**), GATES (**13**), FARMER and MOORE (**9**), and FARMER and SHOVE (**10**) say that the spirem has split in preparation for the homotypic division. In this latter case, however, the split was not traced to its origin. The two halves soon approximate so closely that the spirem appears as a homogeneous structure (figs. 15, 16). Comparatively few ends are seen when sections are cut 12 μ thick, and from all of those observed it appeared to be due to cutting. So far as the stages to follow are concerned, it would make little or no difference whether the spirem be continuous or non-continuous.

At first, as formerly stated, the spirem is irregularly placed within the nuclear cavity (figs. 14, 15), this being followed by a peripheral arrangement, thereby forming the typical hollow spirem as described by MOTTIER (**21, 22**), MOTTIER and NOTHNAGEL (**24**), and BEER (**2**); at which time the thread may be followed for a considerable distance. As it thickens and shortens, the second contraction period is entered upon, although OVERTON (**26**) fails to find such a stage in *Thalictrum purpurascens*, *Calycanthus floridus*, and *Richardia africana;* also GRÉGOIRE (**16**) fails to observe it at times in *Lilium speciosum;* both regarding such an act as of little significance in the reduction division. The author believes this step to be of considerable importance.

Up to and including the second contraction, a nucleolus is usually present (figs. 17, 18), although some of the drawings fail to show such, as it was either not included in that section, or it was purposely left out, owing to obscuring too great a portion of the other chromatin material.

During second contraction (fig. 8), the characteristic radiating loops extend from the tangled mass, the first sign of segmentation being seen at the peripheral end of the loop, resulting in the free ends being next to the nuclear membrane (figs. 19, 20). ALLEN (1) has reported a similar observation in *Lilium canadense*, but this is contrary to the reports of MOTTIER (**21, 22, 23**) and BEER (**2**), these investigators describing the radiating loop as forming the bivalent. With the loop segmenting at its outer bend, it necessarily follows that either the bivalent is continuous at the lower end, as stated in the description of this stage, or that segmentation occurs at both ends, followed by a pairing of single somatic chromosomes. During the period of segmentation and formation of bivalents, the chromatin thread contracts, although not suddenly (cf. figs. 18–24), resulting in 8 thick bivalents being fairly evenly distributed within the nuclear cavity.

All cytologists agree upon the point that in heterotypic mitosis a bivalent consists of 2 somatic chromosomes, but concerning the mode of formation there is a great difference of opinion. ALLEN (1), BERGH (**3, 4**), GRÉGOIRE (**16**), GRÉGOIRE and WYGAERT (**17**), OVERTON (**25, 26**), ROSENBERG (**27**), and YAMANOUCHI (**32, 33**), who claim there is a pairing of somatic chromosomes or spirems in early prophase, state that the bivalent is composed of 2 segments that in the spirem were side by side, reduction therefore occurring by the pairing of 2 spirems; while BEER (**2**), FARMER and MOORE (**9**), FARMER and SHOVE (**10**), and MOTTIER (**21, 22, 23**) demonstrate that it is formed by the twisting about of 2 somatic chromosomes that previously were end to end in the spirem, thus causing a transverse segmentation to be responsible for the reduction. *Allium tricoccum* confirms this latter view, and if the series here given be followed, it will be seen that the arms *b* and *a* of fig. 19 have not arisen from the separation of 2 approximated spirems of a previous stage (fig. 18), yet doubtless these 2 will

by a gradual thickening (figs. 20, 21, 21*x*, *y*, 22) form a bivalent (fig. 22).

Each bivalent, therefore, is composed of 2 somatic chromosomes, presumably maternal and paternal, that previously had an end to end arrangement in the spirem, and which may be open either at both ends or at one end. In the latter case a later segmentation will separate the two. So far as any result that is to follow is concerned, the author conceives it to be of little importance whether it be the one condition or the other. Some claim that the bivalents are always open at both ends, which would necessarily be the case were they derived from a paired spirem; but definite cases have been found where the bivalents were continuous at one end (figs. 22, 23), and in fig. 22, lying under another bivalent, will be seen one in which the two arms have not twisted about each other as yet, but are lying more or less stretched out in the cavity. Under such circumstances the bivalent could not have been formed as GRÉGOIRE (**16**), ALLEN (**1**), and YAMANOUCHI (**32, 33**) have claimed.

To repeat once more, each arm of a bivalent is necessarily of a double nature, owing to the approximation of the two halves of single somatic chromosomes in synapsis, so that in cross-section a bivalent has a tetrad arrangement.

FORMATION OF SPINDLE AND DAUGHTER NUCLEI

Nothing has been found in the literature describing the third contraction or its relationship to spindle formation.

LAWSON (**18, 20**) finds the weft of fibers, these being transformed cytoplasm, outside the nuclear membrane as early as the spirem stage, although in *Allium tricoccum* they are not visible until segmentation.

After carefully studying the paper entitled "Nuclear osmosis as a factor in mitosis" by LAWSON (**20**), and then comparing the same with results found in *Allium tricoccum*, many points of disagreement were encountered. As has been pointed out by FARMER (**8**), LAWSON has used the term "permeable membrane" in describing the nuclear membrane, after which he continues to speak of osmotic systems and exosmosis. In this discussion, when speaking of the membrane in this connection, the term "semipermeable

membrane" will be used. Undoubtedly the nuclear membrane, which is the inner limiting layer of the cytoplasm formed there by the contact of cytoplasm and karyolymph, is a semipermeable membrane, and also, at the time of spindle formation, exosmosis is taking place, since the nuclear cavity is gradually decreasing in size (figs. 22–27) from the time of development shown in figs. 22 and 23, when the fibers are first evident outside the membrane. Since the membrane results from the contact of cytoplasm and karyolymph, the same chemical reaction would occur when the nuclear sap gradually diffuses into the cytoplasm. Such is the author's interpretation of the weft of fibers outside the nucleus. The diffusion is gradual, and the precipitation would then be slight, resulting in the fine fibers. The cytoplasm is not forced to occupy more cubical space, as LAWSON claims, for karyolymph is steadily, though slowly, passing through this semipermeable membrane, and owing to the precipitation the fibers occupy the space left by the receding nucleus.

It is while the foregoing is occurring that the third contraction or massing of the chromosomes in the center of the cavity becomes so conspicuous (figs. 23–27), although as yet the fibers are not in contact with them; and furthermore, the membrane disappears previous to the filling up of the cavity by the fibers (figs. 24–26). If the plasma membrane completely invested the bivalents, as claimed by LAWSON, a cavity would be left between it and the fibers in the case of *Allium tricoccum* (figs. 25, 26), under which circumstances the fibers could not be moored to the membrane (LAWSON **20**).

It was owing to the contact of the two chemically different substances that the heavy plasma membrane was precipitated, but with the kinoplasmic fibers now being formed between the membrane and the reticulate cytoplasm, this chemical antithesis is decreased, resulting in a decrease in the amount of precipitation. In other words, the membrane becomes thinner and thinner until it either disappears entirely or becomes a part of the spindle fibers. When the nuclear cavity is completely filled with the kinoplasmic threads, the chromosomes loosen up, probably owing to the completion of exosmosis from the cavity, as well as partially on account of the osmotic systems within each chromosome.

The bivalents are now open at both ends and not quite as large as formerly, decreasing from now on (fig. 24). When they first loosen up, fibers are not fastened to each chromosome apparently; but as the multipolar complex forms, a weft becomes moored to each one, in many instances so conspicuous (figs. 29–35) that it appears to be homogeneous structure at the point of attachment, owing to the thick fibers and also to their close association.

Each chromosome, as stated by LAWSON (**20**), is saturated with karyolymph and is an osmotic system; although this does not necessarily mean that the old membrane must surround each member in order to accomplish this. If such were the case, the membrane would have to break up into the proper number of pieces, wrap about each chromosome, and then become sealed; a process which is far more complex than ever before attributed to a nucleus. Each chromosome will have the power to develop its own membrane, owing to diffusion of the sap from it, and since each then will have the same osmotic power as the nucleus did as a whole, the same process will continue as it did with the nucleus, resulting in the formation of a weft of fibers from each individual. The fact that these wefts are distinct from the other fibers points strongly toward this idea.

LAWSON'S theory that the shift from multipolar spindle to bipolar spindle is an expression of "lines of tensions" appears to the author to have little or no foundation, since the reticulate cytoplasm is not forced to occupy less cubical space than formerly.

The paired chromosomes, which formerly were tightly twisted about each other, gradually come to lie upon the equatorial plate; the fibers formed outside the nuclear membrane extending from pole to pole and the individual tufts of fibers attached to the chromosomes extending to but one pole. At metaphase the separation of whole somatic chromosomes, that previously were end to end in the spirem, is completed, this reduction being immediately followed by the longitudinal separation of the halves (fig. 35) of each of these, the origin of which was seen in the resting nucleus (figs. 2–10). Those believing in the pairing of somatic chromosomes or spirems in early prophase or pseudo-reduction have little to say concerning the origin of this split; while those claiming that

the spirem is a single structure splitting during the spirem stage demonstrate that the separation during anaphase is the result of this former activity.

Contrary to most reports, the halves of the chromosomes in *Allium tricoccum* become entirely dissociated during anaphase, so that a polar view of such shows 16 individuals, or the *2x* number. These 16 remain distinct from now on, and at late anaphase or early telophase they join up end to end (fig. 38), after which the looping spirem becomes very much crowded. As some karyolymph still remains, this diffuses out and the nuclear membrane is formed about the densely crowded chromatin mass, as described by LAWSON (**20**) and YAMANOUCHI (**32**).

HOMOTYPIC MITOSIS

A spirem consisting of 16 segments, that approximated end to end at late anaphase of the previous mitosis (fig. 38), forms early in the daughter nucleus (fig. 42), since a resting condition does not intervene. In all reports read concerning the homotypic divisions, the spirem is interpreted as being *x* chromosomes in length and double, owing to the longitudinal separation during the previous anaphase. As the multipolar spindle appears, the spirem forms 16 segments (fig. 44) which immediately pair (fig. 45), forming 8 pairs of half chromosomes. It is probably owing to the rapidity of the pairing that previous investigators have claimed the longitudinal split to be completed at this time instead of during the heterotypic mitosis. Unless a close series had been followed and the end to end approximation of the half chromosomes been observed, the author would probably have made a similar interpretation. If each chromosome is to maintain its individuality, no other results could be expected.

All this goes to show that at metaphase of heterotypic division there is a reduction or separation of characters, but it is not until homotypic division that the reduction in number is actually accomplished.

Conclusion

In comparing the nuclei during the reduction division, as seen in *Allium tricoccum*, with the nuclei during somatic division, as described by SHARP (**28**) for *Vicia Faba* root tips, it is seen that

both have a similar structure during the early prophase. The first difference is evident when, apparently owing to some osmotic force, the thread contracts into the synaptic ball. During this contraction there is an increase in chromatin substance, preparatory to the two rapid divisions which follow. At the time of second contraction, this condition undoubtedly being due to osmotic activity again, bivalents are formed. Following the third contraction, where it has been pointed out plainly that exosmosis is the factor underlying spindle formation and massing of bivalents, the members of each pair separate for opposite poles. Seemingly this last contraction, or the 3 contractions taken together, hold in check the dissociation of the halves, which in somatic mitosis would have occurred at this time; for immediately following the separation of whole chromosomes, the halves move apart, join end to end, and, as soon as a new spindle is formed, go to opposite poles.

From this investigation it appears to the author that on account of these various contractions, a regular somatic mitosis, although started normally, is first varied and then checked for a time, resulting in the heterotypic division, and not until homotypic division is the typical mitosis completed.

Summary

1. During late telophase of the last division of the sporogeneous tissue a row of vacuoles appears along the median longitudinal axis of each chromosome, these enlarging until each member is a ladder-like structure. Accompanying this there is an end to end approximation. Such is the condition of the resting nucleus.

2. The paired threads entering synapsis and there approximating are the two sides of the ladder, the connecting strands having broken down. This process does not represent the pairing of two spirems. Throughout this period the chromosomes have retained their individuality.

3. The spirem, which consists of 16 end to end chromosomes, will take the form of radiating loops during second contraction, segmentation occurring at the outer bend. Each of the bivalents so formed consists of two somatic chromosomes that were end to end in the spirem.

4. Spindle fibers are the result of exosmosis of karyolymph into the cytoplasm, these being formed after the same fashion as the nuclear membrane. The membrane, if it persists, will be a part of the fibers.

5. The third contraction which accompanies fiber formation consists of a balling up of the chromosomes previous to the complete filling up of the cavity with fibers.

6. Each chromosome is an osmotic system in itself and capable of forming its own weft of fibers after the sap from the nuclear cavity has been exhausted.

7. The heterotypic division or the reduction of characters results from a transverse separation of whole chromosomes.

8. During early anaphase the halves of the chromosomes, which originated in presynapsis, separate longitudinally and at early telophase approximate end to end, forming the looping spirem of the daughter nucleus, $2x$ chromosomes in length. Division in homotypic mitosis, therefore, results originally from a longitudinal separation.

9. The transverse separation of the 16 segments during early metaphase of the homotypic mitosis is immediately followed by their pairing.

10. To all appearances a typical mitosis is begun, but is varied and delayed for a time by a heterotypic mitosis as the result of the various contractions, being finally completed in the homotypic division.

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UNIVERSITY OF CHICAGO

LITERATURE CITED

1. ALLEN, C. E., Nuclear division in pollen mother cells of *Lilium canadense*. Ann. Botany **29**:189–258. *pls. 6–9*. 1905.
2. BEER, R., Studies in spore development. II. On structure and division of the nucleus in the Compositae. Ann. Botany **26**:705–726. 1912.

3. BERGH, JULES, La formation des chromosomes hétérotypiques dans la sporogénèse végétale. II. Depuis la sporogonie jusqu'au spirème definitif dans la microsporogénèse de *l'Allium fistulosum*. La Cellule **21**: 383–397. 1904.
4. ———, La formation des chromosomes hétérotypiques dans la sporogénèse végétale. III. La microsporogénèse de *Convallaria majalis*. La Cellule **22**:41–53. 1904.
5. BONNEVIE, K., Chromosomenstudien. III. Chromatinreifung in *Allium Cepa* (♂). Arch. Zellforschung **6**:190–253. 1911.
6. DAVIS, B. M., Cytological studies on *Oenothera*. II. Reduction division of *Oenothera biennis*. Ann. Botany **24**:631–653. 1910.
7. DIGBY, L., The somatic, premeiotic, and meiotic nuclear division of *Galtonia candicans*. Ann. Botany **24**:727–758. 1910.
8. FARMER, J. B., Nuclear osmosis and meiosis. New Phytol. **12**:22–28. 1913.
9. FARMER, J. B., and MOORE, J. E. S., The meiotic phase in animals and plants. Quart. Jour. Micr. Sci. **48**:489–555. 1905.
10. FARMER, J. B., and SHOVE, DOROTHY, On the structure and development of the somatic and heterotypic chromosomes of *Tradescantia virginica*. Quart. Jour. Micr. Sci. **48**:559–571. 1905.
11. FRASER, H. C. I., The behavior of the chromatin in the meiotic divisions of *Vicia Faba*. Ann. Botany **28**:633–643. 1914.
12. FRASER, H. C. I., and SNELL, F., The vegetative division of *Vicia Faba*. Ann. Botany **25**:845–855. 1911.
13. GATES, R. R., A study of reduction in *Oenothera rubrinervis*. BOT. GAZ. **46**:1–35. 1908.
14. GRÉGOIRE, V., Les résultats acquirés sur les cinèses des maturation dans les deux règnes. Premier mémoire Revue critique de la littérature. La Cellule **22**:221–376. 1905.
15. ———, Les fondements cytologiques des théories courantes sur l'hérédité Mendelienne. Ann. Soc. Roy. Zool. et Malacol. Belgique **42**:267–320. 1907.
16. ———, La formation des gémini hétérotypique dans les végétaux. La Cellule **24**:369–420. 1907.
17. GRÉGOIRE, V., and WYGAERT, A., La reconstruction du noyau et la formation des chromosomes dans les cinèses somatique. I. Racine de *Trillium grandiflorum* et télophase homotypique dans le *Trillium cernuum*. La Cellule **21**:7–76. 1903.
18. LAWSON, A. A., Studies in spindle formation. BOT. GAZ. **34**:81–100. 1903.
19. ———, The phase of the nucleus known as synapsis. Trans. Roy. Soc. Edinburgh **47**:591–604. 1911.
20. ———, Nuclear osmosis as a factor in mitosis. Trans. Roy. Soc. Edinburgh **48**:137–161. 1911.

21. MOTTIER, D. M., Heterotypic chromosomes. BOT. GAZ. 40:171–178. 1905.
22. ———, The development of the heterotypic chromosomes in pollen mother cells. Ann. Botany 21:309–349. 1911.
23. ———, Mitosis in pollen mother cells in *Acer Negundo* L. and *Staphylea trifolia* L. Ann. Botany 28:115–135. 1914.
24. MOTTIER, D. M., and NOTHNAGEL, MILDRED, The development and behavior of the chromosomes in the first or heterotypic mitosis of pollen mother cells of *Allium cernuum* Roth. Bull. Torr. Bot. Club 40:555–565. 1913.
25. OVERTON, J. B., Über Reduktionsteilung in den Pollen Mutterzellen einigen Dikotylen. Jahrb. Wiss. Bot. 42:121–153. 1905.
26. ———, On the organization of the nuclei in the pollen mother cells of certain plants with especial reference to the permanence of the chromosomes. Ann. Botany 23:19–63. 1909.
27. ROSENBERG, O., Über die Reduktionsteilung im Droserai Neiddel. Stockholms Högs. Bot. Inst. p. 13. 1904.
28. SHARP, L. W., Somatic chromosomes in *Vicia*. La Cellule 29:297–333. 1913.
29. STOUT, A. B., The individuality of the chromosomes and their arrangement in *Carex aquatilis*. Arch. Zellforschung 9:114–141. 1912.
30. STRASBURGER, E., Reduktionstheilung. Sitzungsber. Konigl. Preus. Akad. Wiss. 18:587–614. 1904.
31. ———, Typische und allotypische Kernteilung. Jahrb. Wiss. Bot. 42:1–70. 1905.
32. YAMANOUCHI, S., Sporogenesis in *Nephrodium*. BOT. GAZ. 45:7–31. 1908.
33. ———, Chromosomes in *Osmunda*. BOT. GAZ. 49:1–13. 1910.

EXPLANATION OF PLATES XXVIII–XXX

All figures were drawn with the aid of a Spencer camera lucida with Bausch and Lomb $\frac{1}{12}$ immersion and ocular 12, except figs. 1–11 inclusive, which were drawn with Bausch and Lomb $\frac{1}{16}$ immersion and ocular 12. Magnification of figs. 1–11 inclusive ×3500; all others ×2650. The plates are reduced to two-thirds the original size.

PLATE XXVIII

FIG. 1.—Late telophase of the last division of the sporogenous tissue, showing the anastomoses and the beginning of vacuolization along the median longitudinal axis.

FIG. 2.—A later stage, most of the chromosomes being vacuolate.

FIGS. 3, 4.—Early stages of the pollen mother cells, which consist of ladder-like structures that arose by vacuolization of the chromosomes; some of the chromosomes still entire, owing to unevenness of vacuolization.

FIG. 5.—Typical resting nucleus of the pollen mother cell; chromatin aggregations comparatively large, and the connecting strands very fine.

FIGS. 6, 7.—Somewhat later stages in which the anastomoses are disappearing, connecting strands between the parallel sides becoming very fine in structure, and the sides of the ladder growing more uniform.

FIG. 8.—Late resting stage; the connecting strands disappearing, leaving two parallel daughter spirems.

FIG. 9.—Early synapsis; the two parallel daughter spirems approaching each other and at places approximating.

FIG. 10.—A later stage; the double nature still plainly discernible in many places.

FIG. 11.—Complete synapsis, showing the coiled spirem in which approximation has been completed.

FIG. 12.—Same as above, but magnification not so great.

FIG. 13.—Coming out of synapsis.

FIG. 14.—Late coming out of synapsis, the double row of granules still visible, these being the remains of the larger chromatin aggregations as seen on the ladder-like structures.

FIG. 15.—Spirem; traces of the granules of early prophase showing.

FIG. 16.—Hollow spirem; granules evident and occasionally a split where approximation was not complete.

PLATE XXIX

FIG. 17.—Beginning of second contraction; the spirem very heavy.

FIG. 18.—Second contraction consisting of radiating loops.

FIG. 19.—Segmentation beginning at the outer bend of the loop; *a* and *a'* formerly being continuous, likewise *b* and *b'*; although at this time *a* and *b* are twisting about each other to form the bivalent.

FIG. 20.—Further contraction of the chromatin mass in which the free ends of the bivalents are radiating out from the mass.

FIG. 21.—Loosening up of the bivalents.

FIG. 21*x* and *y*.—Showing the gradual thickening of the bivalents.

FIG. 22.—Eight bivalents evenly scattered within the nuclear cavity in which some are seen to be open at both ends, others closed at one end, while still another, lying under the others, is seen to be stretched out instead of twisted.

FIG. 23.—Beginning of the fiber formation outside the nuclear membrane which accompanies the third contraction; also the disappearance of the nuclear membrane.

FIG. 24.—A slightly later stage; nuclear membrane apparently gone on one side of the nucleus.

FIG. 25.—Later; the fibers in contact with part of the chromosomes; nuclear membrane entirely disappeared; the multipolar complex begun.

FIG. 26.—A later stage.

FIG. 27.—Third contraction complete; fibers have completely filled the nuclear cavity.

FIG. 28.—Multipolar spindle; chromosome aggregation loosening up.

FIG. 29.—Formation of the individual wefts of fibers for each chromosome; the scattering of the bivalents upon the fibers; and a step farther in the transition from multipolar to bipolar spindle.

FIG. 30.—A later stage.

PLATE XXX

FIG. 31.—Bipolar spindle; bivalents stretched out upon the fibers.

FIG. 32.—Polar view of early metaphase.

FIG. 33.—Polar view of metaphase with bivalents peripherally arranged.

FIG. 34.—Metaphase in which the individual wefts of fibers are extending from each chromosome to the pole; also the fibers' massive organization at the point of attachment; to the left is a weft that has been broken from its chromosome.

FIG. 35.—Early anaphase; the longitudinal halves of each somatic chromosome that arose by vacuolation in early anaphase are again becoming apparent.

FIG. 36.—Anaphase; halves of the somatic chromosomes entirely separated.

FIG. 37.—Polar view of the same.

FIG. 38.—Late anaphase; 16 half chromosomes approximating end to end to form a spirem *2x* chromosomes in length.

FIG. 39.—Daughter nucleus after loosening up of chromosomes.

FIG. 40.—Daughter nucleus; irregular net appearance due to the anastomoses.

FIG. 41.—Daughter nucleus; spirem stage in which the fibers of previous mitosis have not disappeared.

FIG. 42.—Daughter nucleus; late spirem in which the outlines of the segments are discernible.

FIG. 43.—A later stage in which the multipolar complex has appeared.

FIG. 44.—Segmentation into 16 segments followed immediately by their pairing.

FIG. 45.—Polar view of metaphase of homotypic mitosis.

FIG. 46.—Late metaphase of the two daughter nuclei.

FIG. 47.—Same, but only one daughter nucleus in plane.

FIG. 48.—Anaphase of homotypic mitosis.

FIG. 49.—Telophase of homotypic mitosis; end to end approximation of 8 chromosomes just previous to organization of granddaughter nucleus.